The efficacy of using Passive Integrated Transponder (PIT) tags without anaesthetic in free-living frogs

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ABSTRACT

The Passive Integrated Transponder (PIT) System was trialed to determine its usefulness as a permanent marking system for *Litoria aurea*. Six *Limnodynastes peronii* were injected with PIT tags and monitored prior to using this identification method in two *Litoria aurea* populations. The appetite and mobility of captive frogs did not appear to be affected by insertion of PIT tags as evidenced by no significant change in their body weight. No mortality related to the tagging method was recorded. At present, 194 free-living *Litoria aurea* have been marked, with no adverse effects experienced during the tagging process.

INTRODUCTION

The ability to mark individuals reliably is considered necessary for most long-term population, ethological and behavioural studies (e.g., Cecil and Just 1978; Keck 1994). Marking techniques vary greatly depending on the size of the species or population, natural history of the organism, and the amount of time and resources available to the researcher (Heyer *et al.* 1994). However, problems such as changes in behaviour or mobility, tag loss, mortality or increased predation may arise when marking organisms, which can limit some studies and compromise research objectives (e.g., Germano and Williams 1993; Golay and Durrer 1994; Joly and Miaud 1989).

Several published methods for marking anurans allow for the identification of individuals (e.g., Donnelly *et al.* 1994; Ferner 1979; Hero 1989; Nace 1982; Robertson 1984). Of these, toe clipping is one of the most widely used techniques (e.g., Ferner 1979; Hero 1989; Martof 1953; Waichman 1992), and has proven useful for long-term capture-recapture studies (e.g., Carpenter 1954; Sinsch 1988, 1992; Tejedo 1988; Turner 1960). However, the technique may increase morbidity and mortality (e.g., Clarke 1972; Golay and Durrer 1994), and toes of surviving individuals often regrow.

Tags have been commonly used, but with varying degrees of success. Jaw tags (Raney 1940), aluminium bird bands (Kaplan 1958), waistbands (Emlen 1968; Kluge 1981), knee tags (Elmberg 1989) and radioactive tags (Karlstrom 1957) have been utilized to examine movements and population size of frogs. However, the use of any tag can lead to friction wounds, skin tearing, snagging on vegetation or tag loss, all of which can cause undue stress to the individual and compromise the research objectives (Heyer *et al.* 1994).

More recent techniques include polymer marking (Wooley 1973), fluorescent marking (Ireland 1991; Taylor and Deegan 1982), branding (Clarke 1971; Daugherty 1976; Thomas 1975), tattooing (Kaplan 1959), pattern mapping (Andreone 1986; Doody 1995) and radioisotope tagging (Semlitsch 1981). Many of these techniques do not satisfy criteria for long-term marking (Stoddart 1970; Woodbury 1956), nor do they always avoid or reduce undue stress or pain. The well being of tagged animals is essential if these focal animals are to be representative of their free-living state.

The use of microchips (Camper and Dixon 1988) alleviates many of the problems inherent in other methods. The Passive Integrated Transponder (PIT) system is a radio-frequency identification tag which consists of an electromagnetic coil, tuning capacitor and microchip encased in glass. Each tag has an external size of 2.2 × 11 mm and is implanted under the skin or in the body cavity. Each PIT tag is encoded with a unique alphanumeric code (close to one trillion codes available), which may be read directly by a hand-held scanner, or sent to a computer port. The transponder derives the energy needed for its operation from the magnetic field generated by the scanner. Since the tag has no power source of its own, it has an unlimited lifespan. The scanner can read the tag from a distance of up to 20 cm, through most materials and spherically from any direction.

Here, I describe a method for marking frogs which can be carried out quickly and easily in field conditions, without the use of anaesthetic.

METHODS

The equipment required to tag frogs consists of PIT tags (supplied in individually packed, sterilized needles), a modified 3 cm^3 plastic syringe, 0.1% iodine solution, cotton gauze,

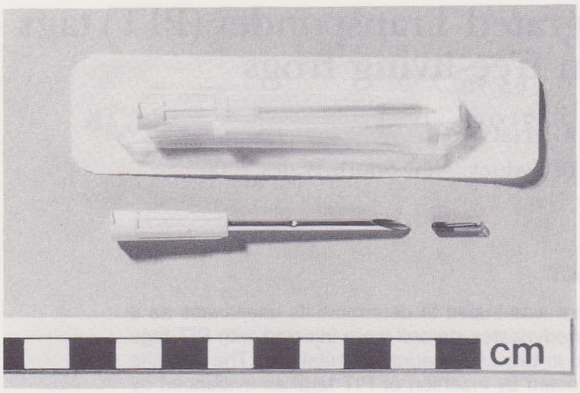

Fig. 1. The 11 mm PIT tags are individually packed in sterilized needles.

medical-grade cyanoacrylate and a PIT tag scanner (Fig. 1).

With one hand, the frog is held ventral side down with the legs held firmly together (Fig. 2). Damp gauze is wrapped around the waist and thigh region to immobilize the frog and maintain the position of the legs. The thumb and forefinger of other hand is used to pin the frog's elbows down and at 90° to the body. Again, damp gauze is used to provide purchase and to maintain moisture to the frog. The palm of the hand is cupped over the frog's head to cover the eyes. This position inhibits the frog's movement during the tagging process, thus decreasing the risk of injury.

Prior to injecting, the lateral skin surface between the arm-pit and the thigh is swabbed using 0.1 % iodine solution as an antibacterial agent. The needle, now attached to the syringe, is inserted subcutaneously just posterior to the axilla facing toward the vent to a depth of approximately 2–3 cm. At this point, the needle should be lying parallel along the dorso-lateral line of the body, and the tag injected by applying pressure to the syringe plunger. The needle is withdrawn slowly, making sure the PIT tag remains *in situ*. Once the needle is withdrawn, forceps are used to hold the wound together while a small drop of medical-grade cyanoacrylate ("Vetbond" supplied by the 3M Group) is applied to close the puncture site. The frog is then placed in a calico bag (lined with plastic and damp paper) for approximately five minutes to recover before being released at the capture site.

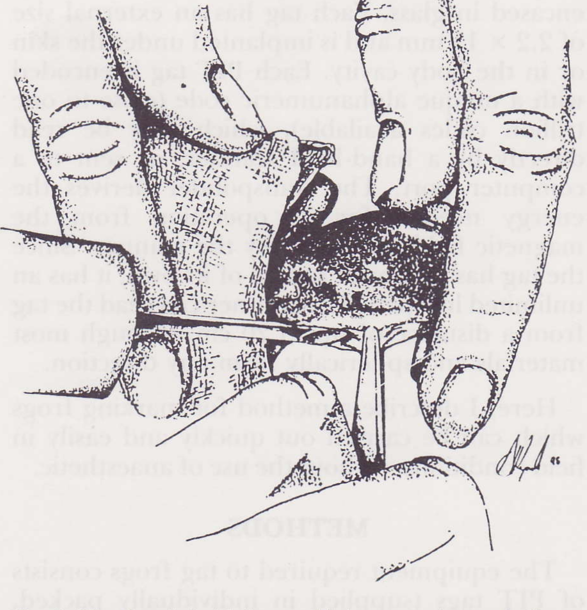

Fig. 2. The frog is held down with legs together and elbows pinned away from the body. The needle is then inserted along the dorso-lateral line of the body.

This procedure was initially tested on six adult *Limnodynastes peronii* in November 1994 (58.4–69.2 mm SVL, 16.2–31.6 g mass). The frogs were housed in individual terraria (approx. 33 × 19 × 23 cm) before being transferred, in pairs, to larger aquaria (40 × 25 × 25 cm) after seven days. Each frog was weighed, measured and sexed (three males, three females) prior to tagging.

RESULTS

The procedure for tagging each frog took approximately five minutes to complete. When held correctly, the frog did not struggle throughout the procedure. Three frogs were observed feeding on crickets within 10 minutes of being tagged and released back into the aquaria, while all frogs consumed four crickets each within 12 hours of tagging. The tissue glue disappeared within two to three days in all cases (n = 6), and the wound site healed completely within three to five days. There was no evidence of infection or trauma at the injection site following tagging.

The frogs were reweighed once a week for the month they were held in captivity, that is, once prior to injecting and three times thereafter. Individuals showed no noticeable change in mass during this period, nor did they display any unusual behaviour or difficulty with mobility or feeding. During this time each male was heard calling on at least one occasion.

Once the trial was complete, the frogs were euthanased and dissected to determine the internal effect of the PIT tags. The internal investigation demonstrated that, in two cases, the PIT tag had attached to the outer peritoneum, while in the remaining four cases the tags continued to "float" within the abdominal cavity.

This technique is currently being used to mark *Litoria aurea* for long-term population and behavioural studies. Since commencing marking *L. aurea* in February 1995, 194 adult frogs (105 females, 89 males) have been successfully

PIT-tagged in the field. Due to the small size of metamorphlings, individuals under 40 mm should not be PIT-tagged until the researcher becomes proficient and confident with the technique.

Thus far, no adverse effects due to the technique have been experienced, with all individuals released without incident. Further, no behavioural changes have been observed in the free-living *L. aurea* population.

The PIT tags and scanners used are marketed by Central Animal Records (Springvale, Victoria, Australia 3172). The prices are approximately $AUD7.00 per tag and $AUD750.00 per minireader scanner.

DISCUSSION

The use of PIT tags to permanently mark individuals is an important break through for long-term population and behavioural studies. PIT tagging eliminates the need for excessive handling following the initial marking session. Individuals can generally be scanned and identified without human contact or disturbance of shelter sites. It should be noted that the PIT system is a marking system, not a locating system. Although the scanner is able to pick up signals through most substances from a distance to approximately 20 cm, some substances are more difficult for the signal to penetrate than others. Thus it is possible that tagged individuals could be difficult to locate unless the substrate covering them is removed and the individual scanned directly.

Since the inert tags are implanted, they cannot snag on vegetation, detach from the individual, fade or potentially attract predators. In addition, the tag is permanent and easy to insert in the field. Most importantly, loss of information due to tag loss, illegible marks and errors derived from applying, reading and recording codes can be overcome using the PIT tag method.

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